Elimination of *Listeria* from a sausage batter by HHP treatment

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Summary: Samples of uninoculated batter of a typical Hungarian fermented pork sausage with or without regular NaCl and nitrite additive, and similar samples but heavily inoculated with *Listeria monocytogenes* have been treated by high hydrostatic pressure (HHP) of 600 MPa for 20 min and reduction of the viable cell count of the test organism was selectively estimated. The HHP treatment caused 5 log-cycles reduction of *Listeria* count in the salt-free batter and around 4 log-cycles in the salt-containing samples. Discolouration of the batter as an effect of the high pressure treatment was observed. It was less pronounced in the nitrite-containing samples. The HHP reduced softness of the batter and changed drastically the pattern of its DSC thermogram pointing for significant protein denaturation.

Keywords: Decontamination, *Listeria monocytogenes,* high pressure

Introduction: Due to its ability.to inactivate vegetative bacterial cells, high hydrostatic pressue above 200 MPa can be one of the alternative non-thermal pasteurization techniques. *Listeria monocytogenes* as an environmental contaminant may contaminate meat trims and it is capable to growth at temperatures of refrigeration and in high-salt environment. Because of the high mortality rate associated with listeriosis, *L. monocytogenes* is given zero tolerance in ready-to-eat meat products in the United States (USDA, 1989). The aim of our studies was to investigate the microbiological efficacy of high hydrostatic pressure to control *L. monocytogenes* in raw batter of a typical Hungarian femented pork sausage. Studies were performed also to clarify the effects of the regualar sodium chloride additive and its curing mixture with nitrite on the high pressure induced changes.

Materials and methods: Pork sausage batter of typical composition (Krommer et al., 2001) has been prepared at the National Meat Research Institute, Budapest. Experimental samples of non-inoculated batter and those, which have been heavily inoculated with a strain of *L. monocytogenes* 4ab before addition of the lactic starter culture, respectively, were divided into small plastic pouches and after sealing under vacuum have been subjected to high pressure treatment of 600 MPa for 20 min in a Stansted "FoodLab 900" type equipment while maintaining them at about room temperature as described by Hassan and others (2002). Analyses were performed immediately after the treatments. Total aerobic viable cell counts (TVC) and *Listeria* counts in inoculated samples were estimated in duplicate samples using plating in Oxford agar with *Listeria* selective supplement. Surface colour by a Minolta tristimulus colorimeter and texture by an SMS TAXT2i type texture analyser were estimated on uninoculated samples. Differential scanning calorimetry (DSC) with a SETARAM "MicroDSC III" microcalorimeter was used to characterize the extent of protein denaturation in HHP treated samples by observing changes in the heat denaturation pattern between 40 and 90 °C.

Results: Uninoculated samples possessed 7.10^5 TVC/g including 2.10^2 bacterial spores/g. The *Listeria* count of the inoculated batter amounted to 5.10^6 cfu/g. The HHP caused somewhat more than 3 log-cycles reduction in the TVC and less than one log reduction in the count of bacterial spores. The *Listeria* count decreased by 5 log-cycles in the salt-free batter, and around 4 log cycles in the salt-containing samples.HHP-treated samples showed increased lightness values and decreased redness values. The yellowness values increased by the pressurization in the salt-free samples but decreased in the

salt-containing ones. The discolouration was less pronounced in the nitrite-containing samples than in the nitrite-less samples. Compressing cylindric samples of presurized sausage batter by the texture analyser to their half-height required approx. 26 % more power in the salt-free samples, approx. 50 % more in the salt containing ones, and 58 % more in those containing both salts than in case of the respective non-pressurized samples. Previously, we have observed reduced softness with correspondingly increased water binding capacity when studied high pressure-induced effects on minced beef (Hassan et al., 2002).This phenomenon was in relation with non-thermal denaturation/coagulation of proteins. Since differential scanning calorimetry is proved to be an effective method to monitor thermal behaviour of proteins related to previous structural changes of meat (Findlay and Barbut, 1990), we have investigated DSC thermograms of untreated and HHP-treated batters.In the thermogram of salt-free, non-pressurized sample a small endothermic peak appeared between 50 and 55 °C, which could be attributed to the heat denaturation of myosin, and myosin subunits, followed by a composite transition between 57 and 68 ^{O}C, showing a distinct large peak and a shoulder which can be considered as heat denaturation of connective tissue (collagen and other stromal proteins) as well as those of sarcoplasmic proteins and the myoglobin. An other distinct peak between 73 and 77 °C was related to the heat denaturation of actin, actinins and troponins (Findlay and Barbut, 1990). The DSC thermograms of HHP-treated samples showed a less complex profile both as the number of heat denaturation endotherms and the enthalpy changes are concerned. These observation showed that most of the proteins of the high-pressure treated samples were in a denatured state in the pressurized batter, except the pressure-resistant collagen. The DSC thermograms of the salt-containing samples showed only one large endotherm at about 65 °C in the unpressurized state, and a greatly diminished one at slightly lower temperature in the pressurized samples.

Conclusions: Our results show that high hydrostatic pressure processing was effective in diminishing considerably *Listeria monocytogenes* inoculated into the raw sausage batter, but it caused some changes in its appearance, texture and protein structure. Discolouration and coagulation of muscle tissues due to HHP are, however, well known (Cheftel and Culioli, 1997). On this basis, further studies are inititated to monitor the fate of surviving microorganisms during fermentation and to assess the quality of the ripened sausage prepared from HHP-pasteurized batter, and to investigate the high pressure effects on electophoretic and immunological cross-reactivity of its proteins (Hajós et al., 2003).

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